U.S. ARMY MEDICAL RESEARCH INSTITUTE OF CHEMICAL DEFENSE

USAMRICD-TR-97-04

Detection and Measurement of Sulfur Mustard (HD) Offgassing from the Weanling Pig Following Exposure to Saturated HD Vapor

Thomas P. Logan
John S. Graham
Jamie L. Martin
John E. Zallnick
Edward M. Jakubowski
Ernest H. Braue, Jr.

November 1997

Approved for public release; distribution unlimited

U.S. Army Medical Research
Institute of Chemical Defense
Aberdeen Proving Ground, MD 21010-5425

DISPOSITION INSTRUCTIONS:

Destroy this report when no longer needed. Do not return to the originator.

DISCLAIMERS:

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

In conducting the work described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," NIH Publication 86-23, revised in 1985.

The use of trade names does not constitute an official endorsement or approval of the use of such commercial hardware or software. This document may not be cited for purposes of advertisement.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (*Leave blank*)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	November 1997	Technical September 1994 - October 1994

4. TITLE AND SUBTITLE
Detection and Measurement of Sulfur Mustard (HD) Offgassing from the Weanling Pig Following Exposure to Saturated HD Vapor

5. FUNDING NUMBERS
62787A
3M162787A872

6. AUTHOR(S)
Logan, TP, Graham, JS, Martin, JL, Zallnick, JE, Jakubowski, EM, and Braue, EH

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research Institute of Chemical Defense
ATTN: MCMR-UV-DB
3100 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5425

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research Institute of Chemical Defense
ATTN: MCMR-UV-RC
3100 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5425

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
USAMRICD-TR-97-04

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (*Maximum 200 words*)

Sulfur mustard (HD) is a chemical warfare agent for which there is neither antidote nor adequate therapeutic protection. Animal models are employed to investigate mechanisms of injury and to evaluate protective measures against HD exposure. Researchers whose experiments involve cutaneous application of HD vapor to animals benefit from the detection and quantitation of HD at the exposed site. The ability to detect and quantify HD enables the researcher to follow safe procedures in handling skin samples. We have designed an experimental procedure to measure HD offgassing from animal models. A Minicams®, which is a portable gas chromatograph (GC) equipped with a flame photometric detector (FPD) and with online sorbent collection and desorption, was used to monitor the HD concentration. Confirming measurements were made using a two-step process that trapped HD on a Tenax sorbent offline and then transferred the sample by means of an ACEM 900 to a GC equipped with either FPD or a mass spectrometer (MS). We collected data from six experiments in which weanling pigs were exposed to saturated HD vapor via vapor caps containing 10 μl of HD. HD concentration was measured in time-weighted-average (TWA) units at a specific HD application site. The currently recommended exposure value for HD is 3 ng/l, 1 TWA unit. In five of the six experiments, Minicams HD concentration values were less than 0.5 TWA, 2 hours postexposure, and in one of the experiments, TWA Minicams concentration was less than 0.5 TWA, 5 hours post-exposure. GC/MS detection was used in three of the experiments to confirm Minicams data and to provide greater sensitivity and selectivity at 0.1 TWA. GC/MS data confirmed that HD concentrations fell below 0.1 TWA in less than 5 hours for a specific site. These measurements of HD concentrations provided information on the expeditious and safe handling of HD-exposed tissue.

14. SUBJECT TERMS
Mustard, HD, Minicams, Gas Chromatography Flame Photometric Detection, Mass Spectrometry, Offgassing

15. NUMBER OF PAGES
23

16. PRICE CODE

S17. ECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UNLIMITED

List of Figures

List of Tables

Introduction

Sulfur mustard (2,2'-dichlorodiethyl sulfide, HD) has been employed as a chemical warfare agent since World War I when its powerful vesicating properties were unleashed at Ypres, Belgium on July 12, 1917. From that day to the present, a huge body of evidence has grown to support the insidious incapacitating capability of HD. Because of this, extensive research has been directed to the protection against, and the treatment of, HD wounds. Part of this chemical defense effort involves the use of animal models. In a typical experiment, saturated HD vapor is applied to the skin of the animal using vapor caps containing 10 μl of HD.[1,2,3] The vapor caps are adhered to the animal for measured time intervals designed to measure specific aspects of the wound such as concentration of HD in tissue,[4] erythema, Nikolsky's sign, and microblister formation.[5] After the vapor caps are removed, the safe handling of the exposed skin site containing HD becomes a decision for the researcher. Previously, when concentration determinations for HD offgassing were not made, a 24-hour delay between agent use and subsequent work outside engineering controls (laboratory safety hoods) was common practice.[6] We provided an analytical procedure based on solid sorbent-gas chromatographic techniques for detecting HD below the recommended time-weighted-average (TWA) value of 3 ng/l.[7] These detection techniques enable the researcher to make informed decisions about the safe handling of exposed animal tissue.[8]

Materials and Methods

1. The sulfur mustard (2,2'-dichlorodiethyl sulfide, HD) employed in this study was lot #HD-U-2325-CTF-N-1, 97.2 mole % (US Army Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground (USAERDEC), MD). Stock solutions of HD in hexane at 9.5 mg/ml were diluted with hexane to 1.9 ng/ ng/μl for calibration of Minicams and GCFPD. USAERDEC, Monitoring Branch provided a stock solution, Aircombo-4249-CTF-Dil, of 1.04 ng/μl of HD in hexane for GCMS calibration.

2. Minicams (O.I. Analytical, CMS Field Products Group, Birmingham, Alabama) measurements were made on a laboratory monitoring system designed 1) to collect HD on Tenax solid sorbent, 2) to thermally desorb and then gas chromatographically separate mixtures and 3) to detect HD by flame photometric detection (FPD) using a sulfur filter. The 5-minute cycle of the Minicams consisted of a 3-minute sampling period, when sample is collected on a preconcentrator tube (100 mm L, 2.4 mm ID, 15 mm bed of Tenax), followed by a 2-minute purge, when desorption and gas chromatographic analysis occurs. A 7 m, 0.32 mm ID, 5 μm DB-1 column (J & W Scientific, Folsom, CA) with a helium flow rate of 20 ml/min produced HD retention times of 91 s when the column was heated from the 30-s to the 90-s point of the cycle at 150°C/min. Both the hydrogen and air flow rates were 35 ml/min for the FPD. Measurements were made continuously in 5-minute cycles with a Minicams by placing a 2 in diameter funnel several mm from an HD-exposed dorsal or ventral site of a weanling pig. During the sampling phase of the cycle, an air sample was drawn at approximately 1 L/min measured with a Mass Flow Meter, model FM-360 (Tylan Corporation, Torrance, CA,) through a heated line onto a preconcentrator tube within the Minicams.

3. Gas chromatographic flame photometric detection (GC/FPD) measurements were made with a Hewlett Packard, Model 5890-Series II, gas chromatograph and Hewlett Packard 19258A flame photometric detector (Hewlett Packard Rockville, MD) on air samples trapped on Tenax tubes (Dynatherm Analytical Instruments, Kelton, PA). The samples were collected at 1 L/min using a vacuum line and flow meter, model B-157-2, (Porter Instrument Co., Hatfield, PA). Air samples were collected by sequentially passing over Tenax, and then bubbling through 5% sodium hypochlorite solutions to assure decontamination of any residual HD. The helium carrier gas flow rate was 4 ml/min through the column, and the flow through the detector was adjusted to 20 ml/min with auxiliary helium. For optimum sulfur detection, hydrogen and air flow rates were 60 ml/min and 100 ml/min respectively with the FPD temperature at 230 °C. The temperature program was initially set at oven temperature 90°C for 1 min, with a 30 °C/min ramp rate to a final temperature of 225 °C that was maintained for 0.5 min. Desorption of samples from Tenax tubes onto a GC column was accomplished through ACEM 900 instrumentation (Dynatherm Analytical Instruments, Kelton, PA). The GC was equipped with a 15 m, 0.53 mm ID, 1.0 μm DB-17 column.

4. Gas chromatographic mass spectrometric detection (GC/MS) measurements were made with a Hewlett Packard, Model 5890 gas chromatograph and Hewlett Packard 5970 mass-selective detector (MSD)- (Hewlett-Packard, Avondale, PA, USA) on air samples trapped on Tenax tube. The samples were collected as described above. The helium carrier gas flow rate was 1 ml/min. The temperature program was initially set at oven temperature 40°C for 1 min, with a 20 °C/min ramp rate to a final temperature of 250 °C that was maintained for 3 min. The transfer line to the MSD was 280 °C. Desorption of samples from Tenax tubes onto a GC column was accomplished through ACEM 900 instrumentation. The GC was equipped with a 30 m, 0.25 mm ID, 0.25 μm HP-5 column. Analyses were made in the selected-ion mode for detection of the ions and fragment ions of HD at m/z 109, 111, 158, and 160.

5. An ACEM 900 for sample concentration and sample focusing was interfaced to Hewlett Packard, Model 5890-Series II, gas chromatographs with either a Hewlett Packard 19258A flame photometric detector or an HP 5970B mass-selective detector. A primary Tenax tube 6 mm o.d. with 4 mm i.d., 110 mm length was used to collect the sample, and a second, lower volume tenax tube (6 mm o.d., 0.9 mm i.d., 185 mm length) was used to focus the sample prior to thermal desorption onto the GC column. Helium continuously flowed through the primary tube at 25 ml/min. A 1 m X 0.20 mm deactivated fused-silica transfer line connected the ACEM 900 to the GC column.

ACEM 900 operating temperatures, when interfaced to the GC/FPD were valve 175 °C, transfer line 250 °C, tube desorb 270 °C, tube idle 60 °C, and trap desorb 300 °C. Time settings were tube dry 1 minute, tube heat 3 minutes, tube cool 1 minute, trap heat 2 minutes, and system recycle 1 minute.

ACEM 900 operating temperatures, when interfaced to the GC/MS were valve 200 °C, transfer line 250 °C, tube desorb 275 °C, tube idle 60 °C, and trap desorb 275 °C. Time settings were tube dry 1 minute, tube heat 2 minutes, tube cool 1 minute, trap heat 2 minutes, and system recycle 1 minute.

6. Calculations of HD concentrations using flame photometric detection in the sulfur mode were made with the equation

$$\text{amount} = m \times (\text{response})^{1/1.83}$$

with amount in nanograms (ng), m is a response factor determined from 1.9 ng/ul standard, and response is area in nanoamp-seconds (na-sec). Figures 1 through 6 were made with GraphPad Prism version 2.00, GraphPad Software, Inc, San Diego, CA. GCMS data and quantitation were made with HP G1034C MS ChemStation Software, Hewlett Packard, Rockville, MD.

Laboratory Animal Procedures

Animal Model

Six male castrated Yorkshire cross weanling pigs, *Sus scrofa*, 7-10 kg, were used (Archer Farms, Belcamp, MD). They were quarantined for seven days and screened for evidence of disease before use. They were maintained under an AAALAC accredited animal care and use program. Animals were supplied tap water *ad libitum* and fed two scoops (equal to approximately 1250 g total) of Lab Porcine Chow Grower (Purina 5084, Purina Mills, Inc., Richmond, IN) twice a day. To prevent interaction and any possible subsequent damage to skin, animals were housed individually in 4 x 6 ft pens with slatted aluminum floors. A small rubber mat was placed on the bottom of each pen to help protect skin lesions from abrasion while the animals slept or recouperated from anesthesia. The animal holding room was maintained at 21° ± 2 °C with 50% ± 10% relative humidity using at least 10 complete air changes per hr of 100% conditioned fresh air. Animal rooms were maintained on a 12-hr light/dark full spectrum lighting cycle with no twilight. Large plastic balls (7" diameter) were placed in the animal runs for environmental enrichment.

Sulfur Mustard Exposure

Eighteen to twenty-four hr before agent exposure, the exposure site areas of pigs were closely shaved first with electric clippers and then with a commercial shaving cream and disposable shaving razor. The following day the animals were anesthetized with a combination of xylazine HCl (2.2 mg/kg, IM) and tiletamine HCl and zolazepam HCl (each at 6 mg/kg, IM), and then placed in either sternal or vertebral recumbency. Heating pads were placed under the animals during agent exposure. The shaved skin of anesthetized weanling pigs was exposed to saturated sulfur mustard vapor for 16 or 36 min. Four animals had 24 dorsal exposure sites and two animals had 12 ventral exposure sites. A plastic template was used for consistent anatomical positioning of the sites. Figures 7 and 8 describe the dorsal and ventral sites, respectively.

The agent challenge was established by varying the duration of exposure to HD vapor generated under 14-mm diameter polyethylene caps (No. 300-1006-020, Evergreen Scientific, Los Angeles, CA). Discs of filter paper were fixed 5 mm above the cap rim against the top inner surface of the caps and wetted with 10 μl of undiluted HD. A previous study[9] established this volume of HD as sufficient to wet the filter paper without run-off. HD-loaded caps were stored

with the rims down on glass microscope slides until application. The vapor caps were held to the skin by double-sided tape assemblies (4.0 x 2.5 cm with a 12-mm hole in the center, thereby creating an exposure area 12 mm in diameter). Although the HD vapor concentration under the caps was not determined, it was estimated from the equilibrium vapor pressure of 0.090 mm of Hg at 30°C to correspond to a vapor concentration of 1.4 mg/l.[10] After removal of the caps and tape assemblies at the appropriate times, the animals were placed in individual holding cages (24" L x 18" W x 16" H) under a connecting hood for 24 hr. Air samples were taken from HD exposed sites for GC analysis during this time. No signs of discomfort or pain were noted during the experiment. Reports from studies in human volunteers indicate that exposure of forearm skin to 10 min of HD vapor is "practically painless" with any later discomfort due to the lesions themselves and not the sulfur mustard.[11] Following the 24-hr holding period, the pigs were moved back to their large pens in the animal holding room for the duration of the experiment.

Results and Discussion

Figures 1 through 6 show the Minicams concentration of HD at an HD-exposed site versus time in minutes with data points collected at 5-minute intervals. Concentrations are expressed as 8-hour time-weighted-average (TWA) concentrations. The currently recommended exposure value for 1 TWA unit for HD is 3 ng/l.[5] Figure 1 contains the first experimental measurement of HD detected from pig 1 at site 19. In this initial experiment, sites 16 and 24 were also investigated. The sudden increase of TWA levels in Figure 1 was attributed to animal movement between data points 5 and 6 and change of sampling from site 19 to site 16 for data point 6. Figure 2 contains data from pig 2 at site 15 with offgassing measurements of HD remarkable for their sudden fluctuations. Pig 2 required the most anesthesic injections of any animal in this 6-animal study as indicated in Table I. The erratic profile in Figure 2 was attributed to pig 2 being the most active of the six pigs; this activity resulted in contractions and expansions at HD-exposed sites causing fluctuations of HD offgassing from reservoirs of active HD.[12] Pig 2 was spiking concentrations above 1 TWA almost 5 hours postexposure. Figure 3 contains data from pig 3 at site 7, and the most notable change is the sudden increase in HD concentration between data point 3 and 4. The increase in TWA at the fourth data point in Figure 3 corresponded to a correction of sampling position on site 7 at this point in the experiment. Figure 4 contains data from pig 4 at site 8; Figure 5 contains data from pig 5 at site 23; Figure 6 contains data from pig 6 at site 18. Figures 4, 5, and 6 show a relatively continuous decrease in TWA with time which demonstrates improved sampling conditions and procedures for pigs 4, 5, 6. For five offgassing studies the Minicams concentration was below 0.5 TWA within two hours of exposure and for one study, pig 2, 5 hours and 5 minutes were required for concentration to be permanently less than 0.5 TWA.

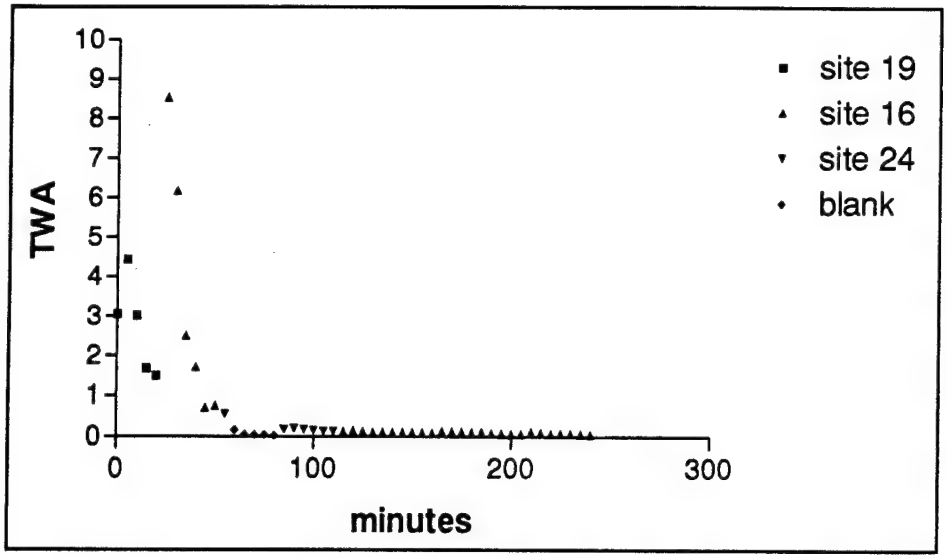
Tables II, III, and IV present TWA values obtained by the three different analytical methods: Minicams, GC/FPD (Figure 9), and GC/MS (Figure 10). Continuous Minicams sampling at neighboring sites contributed to lowering of HD initially detected by the GC/FPD and GC/MS in Tables II and IV. The large initial TWA obtained by GC/FPD in Table III was due to acquiring sample before Minicams sampling began. Continuous Minicams measurements produced a slow, gradual approach to 0.1 TWA. The occurrence of background contamination was attributed to the continuous sampling procedure used with the Minicams. In Tables II-IV, the more rapid

approach to 0.1 TWA concentration by GC/FPD vs Minicams detection was due to 1) the use of one Dynatherm tenax tube per sample with each tube being completely desorbed by heating under nitrogen gas prior to sampling and 2) improvement in GC conditions with a longer column and a lower temperature ramp rate. The GC/MS data confirmed the presence of HD and was able to distinguish HD from peaks that, if present, would interfere with HD when analyzed by GC/FPD. For this reason, the GC/MS data was most reliable, especially at concentrations approaching 0.1 TWA.

Summary

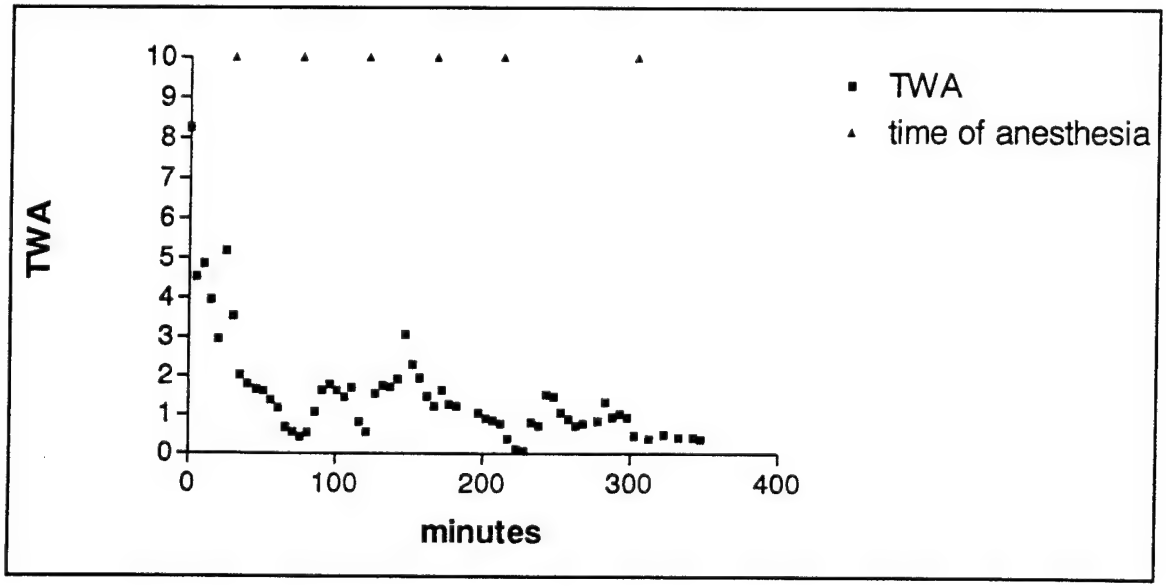
This study provided a quantitative measure of HD offgassing from sites on the weanling pig immediately after exposure. Continuous Minicams measurements in Figures 1-6 indicate site at which specific HD-TWA concentrations were below 0.5 TWA, 5 hours and 5 minutes after exposure for the most active animal, pig 2, while the remaining 5 pigs reached this point in 2 hours or less. At 0.5 to 0.1 TWA the continuous sampling procedure used with the Minicams caused background buildup to interfere with HD offgassing measurements. Selective individual GCFPD detection reached lower HD-TWA concentrations more quickly than continuous sampling with Minicams because of individual sampling tubes and improved gas chromatographic (longer GC column and lower temperature program ramp rate) conditions. Site specific GC/MS data in Tables II, III, and IV confirmed that it required as long as 5 hours to reach 0.1 TWA. We demonstrated that the minicams provided reliable continuous concentration measurement for HD offgassing to 0.5 TWA. We conclude, using 0.1 TWA as a safe concentration, that animals can be safely removed from engineering control 6 hr after the HD exposure we described.

Figure 1



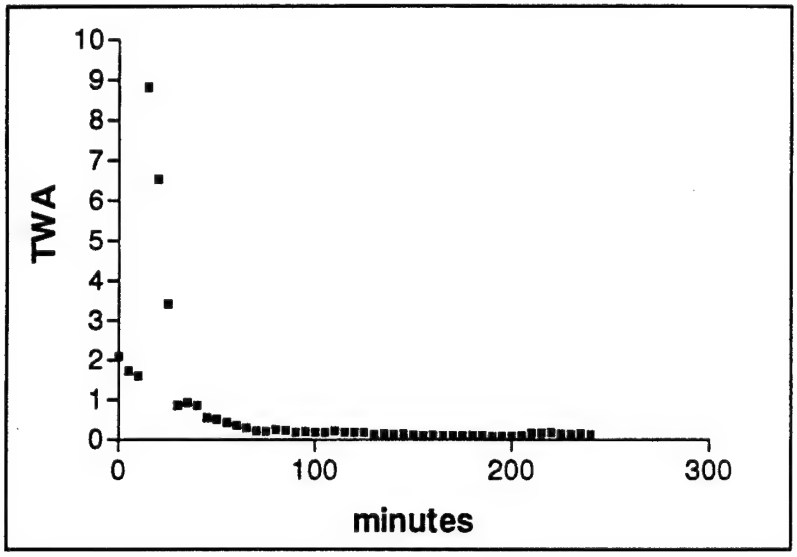
Minicams HD Concentration as Time-Weighted-Averages (TWA) Measured at Sites 19, 16, and 24 on Pig 1.

Figure 2



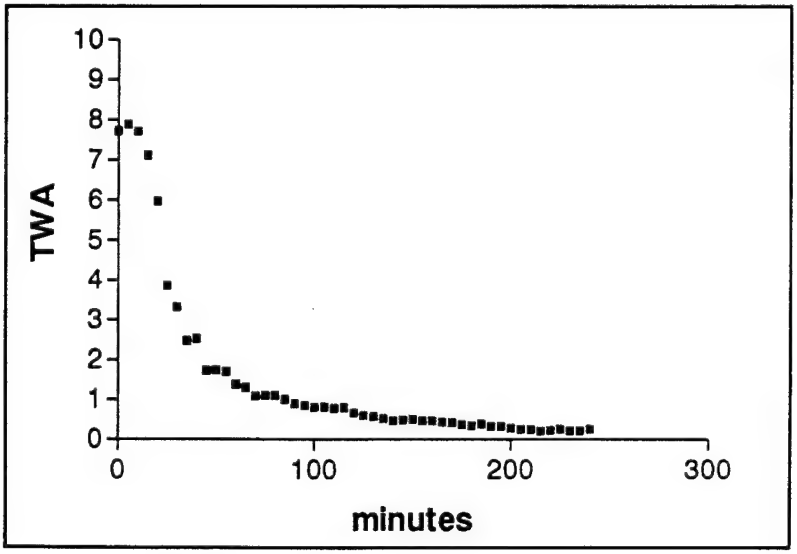
Minicams Measurement of HD Concentration as Time-Weighted-Averages (TWA) from Site 15 on Pig 2. Times of analgesic injection are shown at the top of the figure.

Figure 3



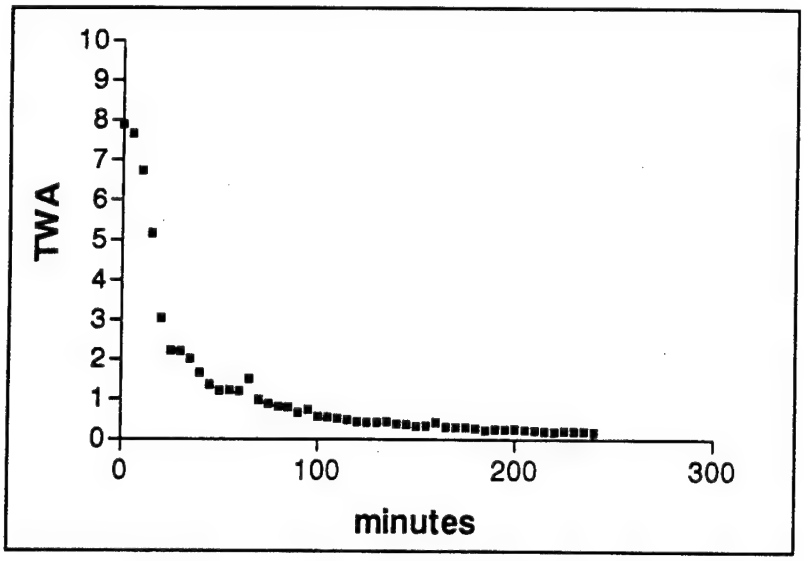
Minicams Measurement of HD Concentration as Time-Weighted-Averages (TWA) from Site 18 on Pig 6.

Figure 4



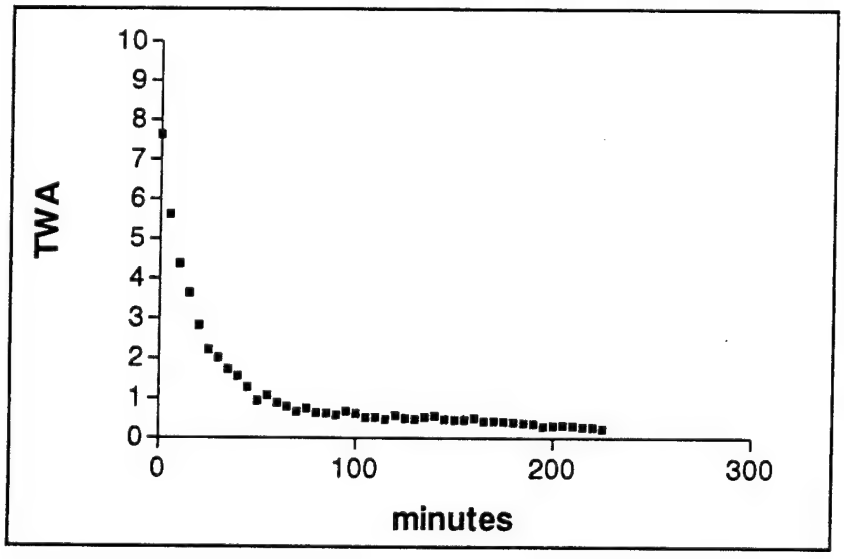
Minicams Measurement of HD Concentration as Time-Weighted-Averages (TWA) from Site 8 on Pig 4.

Figure 5



Minicams Measurement of HD Concentration as Time-Weighted-Averages (TWA) from Site 23 on Pig 5.

Figure 6



Minicams Measurement of HD Concentration as Time-Weighted-Averages (TWA) from Site 18 on Pig 6.

Figure 7

1	2	3	4	5	6
7	8	9	10	11	12

Head----------Dorsal Midline----------Tail

13	14	15	16	17	18
19	20	21	22	23	24

Sites of HD Vapor Cap Dorsal Application on Pigs 1, 2, 5, and 6.

Figure 8

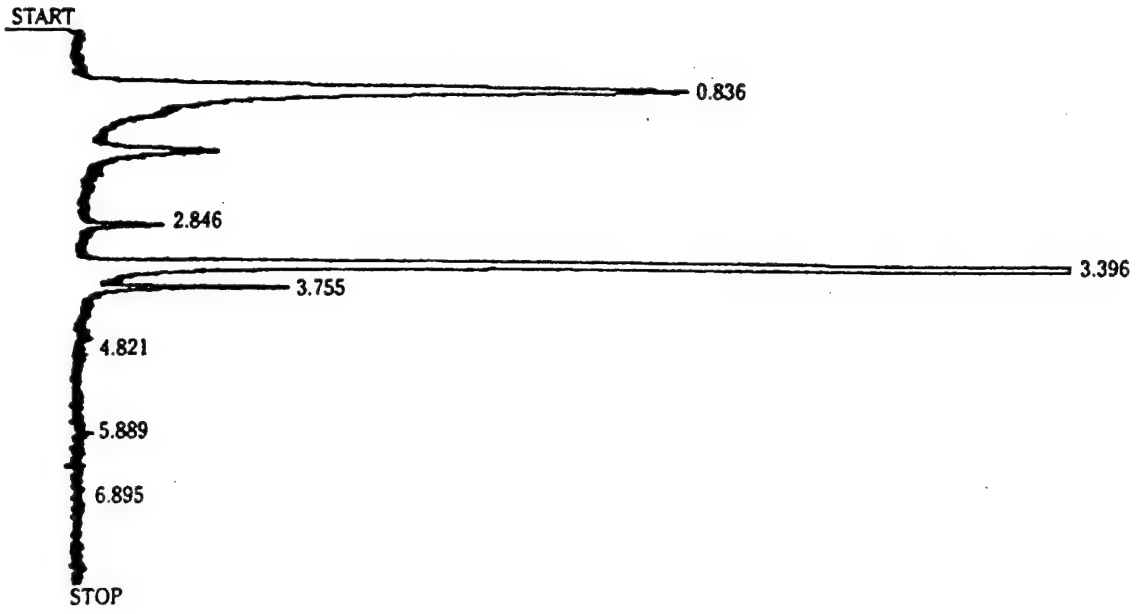
1	2	3
4	5	6

Head----------VerticalMidline----------Tail

7	8	9
10	11	12

Sites of HD Vapor Cap Ventral Application on Pigs 3 and 4.

Figure 9



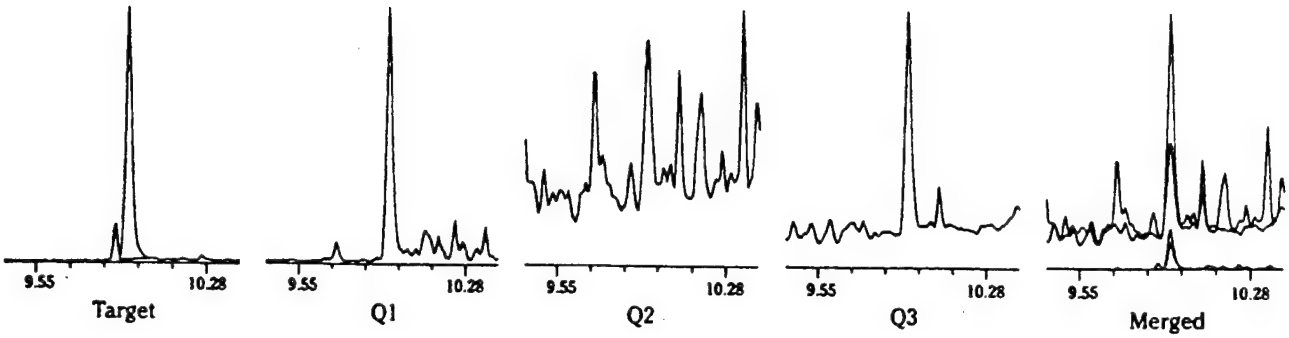
Area%

RT	Area	Type	Width	Area%
.836	212918	BV	.182	10.19699
1.735	12390	VV	.053	.59338
1.755	15566	VV	.058	.74548
2.846	10860	VV	.066	.52010
3.396	1810641	PB	.036	86.71456
3.755	15882	PP	.043	.76062
4.821	1151	VV	.037	.05512
5.662	1935	VP	.059	.09267
5.772	1759	PV	.051	.08424
5.889	2382	VP	.052	.11408
6.895	2563	PB	.087	.12275

Total Area = 2088047
MUL Factor = 1.0000E+00

Chromatogram from Gas Chromatographic Flame Photometric Detection of HD (3.396 min) from Tenax sorbent tubes and transferred by the ACEM 900.

Figure 10



	Signal	Ratios	Limits		RT	Limits	Resp	Integ Type
Tgt	158.00	100.0%			9.94	9.67	114976	sys def
Q1	160.00	66.2	53.9-	80.9	9.94	to	76102	sys def
Q2	111.00	297.6	240.0-	360.0	9.94	10.16	342129	sys def
Q3	109.00	571.4	431.3-	646.9	9.93		656987	sys def

0 x^2 + 97591.1 x + -41192.9
Coefficient is 0.997199

Data from Gas Chromatographic Mass Spectrometric Detection using selected ion monitoring of masses: 158, 160, 111, and 109 atomic mass units (amu) to detect HD from Tenax sorbent tubes and transferred by the ACEM 900.

Table I

Pig	1	2	3	4	5	6
weight kg	9.9	10.5	9.8	7.2	8.8	12.4
anesthesia times	09:26 10:35 11:54	11:04 11:52 12:35 13:19 14:07 15:40	11:10 12:32 13:38 14:14	10:31 13:02 15:59	10:52 11:53 13:43	12:04 13:30 14:51
HD area total sites	dorsal 24	dorsal 24	ventral 12	ventral 12	dorsal 24	dorsal 24
minicam site # monitored	19,16,&24	15	7	8	23	18
HD vapor duration	16	16	16	16	16	36
time vap cap removed	10:12,30 site 16	10:19,20 site 15	10:34,17 site 7	10:03,20 site 8	09:51,40 site 23	11:22,50 site 18
first minicams time	10:14	10:35	10:48	10:12	10:08	11:38
interval in minutes	1(partial first cycle)	16	14	9	16	15

Experimental conditions in the six offgassing experiments.

Table II

time after vapor cap removal	Minicams TWA site 8	time after vapor cap removal	GC/FPD TWA site 10	time after vapor cap removal	GC/MSD TWA site 10
00:23:40	7.12	00:23:30	0.68	00:20:30	0.94
00:28:40	5.98			00:26:30	0.27
01:19:40	1.1			00:19:30	0.02
01:39:40	0.91	01:39:30	0.11		
02:20:40	0.58	02:21:30	0.17		
03:10:40	0.35	03:08:30	0.13		
03:35:40	0.21	03:43:30	0.04		
06:38:40	0.26	06:39:30	0.05		

TWA measured at site 8, Pig 4 by Minicams in column 2, TWA measured at site 10, Pig 4 by ACEM 900-Gas Chromatograph Flame Photometric Detection in column 4, and ACEM 900-Gas Chromatograph Mass Spectroscopic Detection in column 6.

Table III

time after vapor cap removal	Minicams TWA site 23	time after vapor cap removal	GC/FPD TWA site 20	time after vapor cap removal	G/MS TWA site 20
00:16:20	7.9	00:13:50	10.32	00:20:50	1.74
01:01:20	1.37	01:03:50	0.51		
01:11:20	1.23	01:09:50	0.85		
01:17:20	1.2	01:18:50	0.66		
01:57:20	0.57	01:59:50	1.68		
02:27:20	0.42	02:26:60	0.93		
02:58:20	0.32	02:56:50	0.54		
03:48:20	0.22	03:47:50	0.28	03:42:50	0.25
04:34:20	0.19	04:23:50	0.14		
04:39:20	0.14	04:30:50	0.07	04:28:50	0.02
05:20:20	0.14	05:12:50	0.1		
05:45:20	0.17	05:34:50	0.09		
06:05:20	0.17	05:53:50	0.15		
06:46:20	0.15	06:38:50	0.11		
07:16:20	0.14	07:07:50	0.05		

TWA measured at site 23, Pig 5 by Minicams in column 2, TWA measured at site 20, Pig 5 by ACEM 900-Gas Chromatograph Flame Photometric Detection in column 4, and ACEM 900-Gas Chromatograph Mass Spectroscopic Detection in column 6.

Table IV

time after vapor cap removal	Minicams TWA site 18	time after vapor cap removal	GC/FPD TWA site 24	time after vapor cap removal	GC/MS TWA site 24
00:16:00	7.2	00:17:00	2.08		
00:37:00	2.77	00:34:00	0.85		
01:02:00	1.26	00:59:00	0.52		
01:22:00	0.77	01:21:00	0.3		
02:03:00	0.52	02:04:00	0.18		
02:53:00	0.48	02:52:00	0.21		
03:19:00	0.43	03:15:00	0.14	03:21:00	0.09
03:44:00	0.39	03:42:00	0.17		
03:54:00	0.31	03:55:00	0.13		
04:09:00	0.36	04:10:00	0.13		
04:40:00	0.28	04:38:00	0.17		
06:31:00	0.20	06:31:00	0.08		
11:39:00	0.17	11:32:00	0.13	22:45:00	0.05

TWA measured at site 18, Pig 6 by Minicams in column 2, TWA measured at site 24, Pig 6 by ACEM 900-Gas Chromatograph Flame Photometric Detection in column 4, and ACEM 900-Gas Chromatograph Mass Spectroscopic Detection in column 6.

References

1. Marlow D.D., Mershon M.M., Mitcheltree L.W., Jaax G.P., and Petrali J.P. J Toxicol. Cutaneous Ocul. Toxicol. **9**:179-192, 1990.

2. Mershon M.M., Wade J.V., Mitcheltree L.W., Petrali J.P., and Braue E.H., Jr. Fundam. Appl. Toxicol. **15**:622-630, 1990.

3. Braue E.H., Koplovitz I., Mitcheltree L.W., Clayson E.T., Litchfield M.R., and Bangledorf C.R. Toxicol Methods **2**:242-254,1992.

4. Logan, Thomas P., Bongiovanni, Rodolfo, Millard, Charles B., Shutz, Michael B., Schultz, Susan M., Lee, Robin B. USAMRICD-TR-96-04. U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD, October 1996, UNCLASSIFIED. ADA323919

5. Graham, John S., Braue, Ernest H., Jr., Martin, Jamie L., Zallnick, John E., Nalls, Catherine R., and Mitcheltree, Larry W. Assessment of cutaneous sulfur mustard injury in the weanling pig. In: Proceedings of the 1996 Medical Defense Bioscience Review, vol. II, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD, 695-704, 1996. ADA321840

6. Casole, B. Personal Communication

7. Department of the Army Pamphlet 40-173, Medical Services, Occupational Health Guidelines for the Evaluation and Control of Occupational Exposure to Mustard Agents H, HD, and HT, Headquarters Department of the Army, Washington, DC, 30 August 1991.

8. Braue E.H., Jr, SOP #91-067-DB-01, Surety Procedure for Cutaneous Applications of Sulfur Mustard (HD) on the Skin of Laboratory Animals, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD, Jan 1997.

9. Merson M.M., Mitcheltree L.W., Petrali J.P., Braue E.H. and Wade J.V., Hairless guinea pig bioassay model for vesicant vapor exposures. Fund. Appl. Toxicol. 15:622-630, 1990.

10. Redemann C.E., Chaikin S.W. and Fearing R.B., The volatility and vapor pressure of eight 2-chloroethyl alkyl (or cycloalkyl) sulfides, J. Amer. Chem. Soc. 70:631-633, 1948.

11. Daily L.E. and Clark J.W., Naval Research Laboratory, NRL Report No. P-2364, 1944.

12. Renshaw B., Mechanism in Production of Cutaneous Injuries by Sulfur and Nitrogen Mustards. In: Chemical Warfare Agents, and Related Chemical Problems, Report of Division 9, National Defense Research Committee. Washington, DC, 1946, Pts. 3-6, Ch 23.

DISTRIBUTION LIST

Addresses	Copies
DEFENSE TECHNICAL INFORMATION CENTER ATTN DTIC OCP 8725 JOHN J KINGMAN RD STE 0944 FT BELVOIR VA 22060-6218	2
COMMANDER US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND FORT DETRICK MD 21702-5012	2
HQDA DASG HCD WASHINGTON DC 20310	1
DIRECTOR WALTER REED ARMY INSTITUTE OF RESEARCH BUILDING 40 WASHINGTON DC 20307-5100	1
COMMANDER US ARMY AEROMEDICAL RESEARCH LABORATORY ATTN SCIENTIFIC INFORMATION CENTER PO BOX 577 FORT RUCKER AL 36362-5000	1
COMMANDER US ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES BUILDING 1425 FORT DETRICK MD 21702-5011	1
COMMANDER US ARMY RESEARCH INSTITUTE OF ENVIRONMENTAL MEDICINE BUILDING 42 NATICK MA 01760-5007	1
COMMANDANT US ARMY CHEMICAL SCHOOL ATTN ATZN CM C FORT MCCLELLAN AL 36205	1
DIRECTOR ARMED FORCES MEDICAL INTELLIGENCE CENTER FORT DETRICK MD 21702-5004	1
COMMANDER US ARMY INSTITUTE OF DENTAL RESEARCH BUILDING 40 WASHINGTON DC 20307-5300	1
COMMANDER US ARMY INSTITUTE OF SURGICAL RESEARCH BUILDING 2653 FORT SAM HOUSTON TX 78234-6200	1
COMMANDANT ACADEMY OF HEALTH SCIENCES US ARMY ATTN HSHA CDC FORT SAM HOUSTON TX 78234-6100	1
COMMANDANT ACADEMY OF HEALTH SCIENCES US ARMY ATTN HSHA CDM FORT SAM HOUSTON TX 78234-6100	1
DIRECTOR ENVIRONMENTAL AND LIFE SCIENCES OFFICE OF THE DEPUTY DIRECTOR FOR RESEARCH AND ENGINEERING ROOM 3D129 WASHINGTON DC 20301-2300	1
COMMANDER US ARMY TRAINING AND DOCTRINE COMMAND ATTN ATMD FORT MONROE VA 23651	1

COMMANDER 1
US ARMY NUCLEAR AND CHEMICAL AGENCY
7500 BACKLICK ROAD
BUILDING 2073
SPRINGFIELD VA 22150-3198

EXECUTIVE OFFICER 1
NAVAL MEDICAL RESEARCH INSTITUTE
NAVAL MEDICINE COMMAND
NATIONAL CAPITAL REGION
BETHESDA MD 20814

DEPARTMENT OF THE NAVY 1
NAVAL POSTGRADUATE SCHOOL
DUDLEY KNOX LIBRARY
411 DYER ROAD
ROOM 110
MONTEREY CA 93943-5101

USAF ARMSTRONG LABORATORY/CFTO 1
SUSTAINED OPERATIONS BRANCH
BROOKS AFB TX 78235-5000

DEPARTMENT OF HEALTH AND HUMAN SERVICES 1
NATIONAL INSTITUTES OF HEALTH
THE NATIONAL LIBRARY OF MEDICINE
SERIAL RECORDS SECTION
8600 ROCKVILLE PIKE
BETHESDA MD 20894

STEMSON LIBRARY 1
ACADEMY OF HEALTH SCIENCES
BUILDING 2840 RM 106
FORT SAM HOUSTON TX 78234-6100

US ARMY RESEARCH OFFICE 1
ATTN CHEMICAL AND BIOLOGICAL SCIENCES DIVISION
PO BOX 12211
RESEARCH TRIANGLE PARK NC 27709-2211

AFOSR/NL 1
BUILDING RM A217
BOLLING AFB DC 20332

COMMANDER 1
US ARMY CHEMICAL BIOLOGICAL DEFENSE AGENCY
ATTN AMSCB CI
ABERDEEN PROVING GROUND MD 21010-5423

LTC RICHARD R. STOTTS 1
BATTELLE MEMORIAL INSTITUTE JM 3
505 KING AVENUE
COLUMBUS OH 43201-2695

COMMANDER 23
US ARMY MEDICAL RESEARCH INSTITUTE OF CHEMICAL DEFENSE
3100 RICKETTS POINT ROAD
ATTN MCMR UV ZA
MCMR UV ZB
MCMR UV ZS
MCMR UV RC (5 copies)
MCMR UV R (11 copies)
MCMR UV AI W
MCMR UV D
MCMR UV P
MCMR UV C
ABERDEEN PROVING GROUND MD 21010-5425